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Published on Web 01/27/2005

Solutes Probe Hydration in Specific Association of Cyclodextrin and Adamantane

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Abstract: Using microcalorimetry, we follow changes in the association free energy of β-cyclodextrin (CD) with the hydrophobic part of adamantane carboxylate (AD) due to added salt or polar (net-neutral) solutes that are excluded from the molecular interacting surfaces. Changes in binding constants with solution osmotic pressure (water activity) translate into changes in the preferential hydration upon complex formation. We find that these changes correspond to a release of 15−25 solute-excluding waters upon CD/AD association. Reflecting the preferential interaction of solute with reactants versus products, we find that changes in hydration depend on the type of solute used. All solutes used here result in a large change in the enthalpy of the CD−AD binding reaction. In one class of solutes, the corresponding entropy change is much smaller, while in the other class, the entropy change almost fully compensates the solute-specific enthalpy. For many of the solutes, the number of waters released correlates well with their effect on air−water surface tensions. We corroborate these results using vapor pressure osmometry to probe individually the hydration of reactants and products of association, and we discuss the possible interactions and forces between cosolute and hydrophobic surfaces responsible for different kinds of solute exclusion.

Introduction

Celebrated for their unique ability to enhance the solubility of nonpolar "hydrophobic" organic solutes, naturally produced cyclodextrins (CDs) find use in such diverse fields as pharmaceutical, cosmetic, and food industries.[1−8] Shaped as a hollow truncated cone, this cyclic carbohydrate is unique in that it can incorporate nonpolar "guest" molecules in its central cavity to form noncovalent "guest−host" inclusion complexes. Because CD is quite soluble in water, the inclusion complex confers this property on the less soluble guest. Depending on both the CD derivative and host molecule, the association constants for the inclusion complex are typically 10^3-10^5 M^{-1}. Three types of cyclodextrin are naturally most abundant: α-, β-, and γ-CD, containing 6, 7, and 8 α-D-glucose units, respectively. In addition to these naturally occurring species, many synthetic modifications of CD have been produced to increase water solubility and association specificity.

Because CD specifically incorporates nonpolar hydrophobic solutes, it is also attractive as a model system for hydrophobic interactions and their role in determining specificity in biologically relevant systems, such as specific protein−ligand interactions.[9−12] Guest−host association must be accompanied by a release of surface/cavity neighboring waters. Crystal structures show that at full hydration, the cavity of β-CD (on which we focus here) accommodates ≈11 water molecules;[13,14] a similar number can be expected to be released upon association as the guest displaces some or all of these waters.[15] Moreover, a large interacting guest must also shed some or all of its surface waters in order to complex.

More evidence for the anticipated water release comes from the heats of complexation. These measurements show that the CD's interaction with guest molecules has a negative heat capacity, often related to burial of nonpolar surfaces.[16−19] Furthermore, with different solvent conditions and guest molecules, changes in reaction free energy often show "entropy−enthalpy compensation".[20−26] The large residual entropic term,

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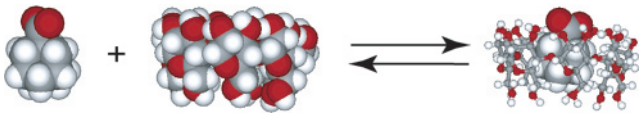

Figure 1. Schematic of the complexation reaction between adamantane carboxylate and β-cyclodextrin. Drawn to scale, the figure shows the close fit of AD in the CD cavity, requiring the release of surface-hydrating waters from both interacting molecular surfaces.

expected (through extrapolation) to persist even in the (hypothetical) absence of heat release, was proposed to indicate water release upon association.[22]

We study the complexation of CD with adamantane carboxylate (AD) acting as a guest. Due to its charged group, AD is water soluble, despite its substantial globular hydrophobic region. The β-CD•AD inclusion complex, shown schematically in Figure 1, is highly favorable, with an association constant of $K_a \approx 5 \times 10^4$ M^{-1} at room temperature. The size of AD is almost exactly accommodated by the cavity of β-CD. NMR experiments show that once complexed, AD fits deep in the CD cavity, with the charged carboxylate group exposed to solution at the wider opening of the host CD.[27]

There are many definitions for waters of "hydration" depending on the probing technique and the energetic or structural criteria applied.[28−35] We use the response to osmotic stress as an operational definition of hydration.[36−39] Many salts and polar solutes are preferentially excluded from hydrophobic surfaces.[40−43] The solubility of many hydrophobic compounds, for example, decreases with increasing concentration of salts or polar solutes due to this unfavorable interaction.[44] A common feature of this exclusion is that the apparent number of solute excluding waters does not vary much with salt or polar solute concentration. The number of these preferentially bound waters, however, sensitively depends on the chemical nature and size of the probing solute as well as on the nature of the macromolecular surface.[37,41−43,45−48] Changes in the number of the preferentially bound waters accompanying binding reactions can be measured from the change in binding free energies ($\Delta G°$) with solute concentration.[37,40,49−51] For a constant difference in the number of included waters, $\Delta G°$ will vary linearly with solute osmotic pressure.

To probe the changes in preferential hydration involved in the CD/AD association, we use two different experimental approaches. The two approaches give a complementary and consistent picture of water release upon complex formation.

In the first approach, we use microcalorimetry to determine equilibrium constants for complexation in the presence of an additional solute (cosolute). We find that for a wide range of cosolutes, the association free energy varies linearly with water chemical potential (or osmotic pressure). This translates into a constant change in the number of cosolute-excluding waters in complexation. However, we find that this difference in numbers of released waters depends on the type of cosolute probing the reaction, reflecting the preferential interaction of cosolutes with (or extent of exclusion from) the complexing molecules.

Using calorimetry, we can also follow individually changes in heats ($\Delta H°$) and entropies ($T\Delta S°$) of association. We find that all cosolutes we have used belong to one of two classes. The first class has a strong enthalpic and smaller entropic contribution to the binding free energy. The second class has a strong enthalpic contribution to the free energy that is almost completely compensated by the entropic change. The dissection of free energy changes into the enthalpic and entropic contributions enables us to discuss the possible intermolecular forces responsible for the different preferential interactions between cosolutes and CD/AD.

In the second experimental approach, we determine the preferential hydration of the individual molecular species using vapor pressure osmometry of CD and AD solutions with added cosolutes. The number of solute-excluding waters associated with each species can be determined from changes in solution osmolalities (osmotic pressures) of mixed solute−cosolute solutions as developed by Courtenay et al.[41,52] Evaluated differences between cosolute-excluding waters of reactants (CD or AD) and products (CD•AD complex) are then simply translated into the changes in hydration upon complexation.

Results and Analysis

Changes in Molecular Association from Calorimetry. Using isothermal titration calorimetry (ITC), we follow the change in heat release, Q, with each injection of CD into a solution of AD. Figure 2 shows three typical plots of the heat release per mole of CD solution, integrated for each injection. The experiment is repeated in the presence of cosolute (glycine in Figure 2) in both CD (injectant) and AD (cell) solutions at the same molal concentrations. One prominent effect of glycine addition is to increase the amount of heat released in the CD/AD association, as seen in the value of heat release for the first injections in Figure 2.

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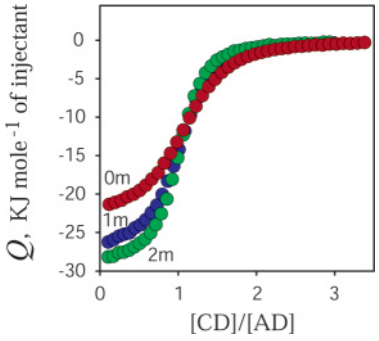

Figure 2. Effect of glycine (cosolute) concentration on heat release in CD/AD complexation measured using ITC. The heat release for each injection of CD solution into AD is shown for three molal concentrations of glycine: 0 m (red), 1 m (blue), and 2 m (green). All measurements were made at 30 °C.

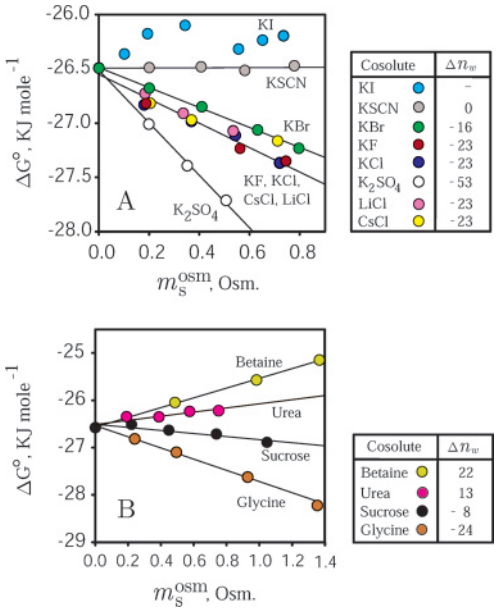

Figure 3. Changes in the number of cosolute-excluding waters (Δn_w) in CD/AD association evaluated from changes in association free energies ($\Delta G°$) with changes in cosolute osmolal concentration (m_s^{osm}) for β-CD (eq 1) with different (A) salts and (B) neutral cosolutes. All measurements were made at 30 °C.

The plots in Figure 2 can be well fit by a 1:1 CD/AD binding model, from which we determine the free energy ($\Delta G°$) of complex formation, AD + β-CD ⇌ β-CD•AD. Figure 3 shows $\Delta G°$ versus the solute osmotic pressure expressed in terms of osmolal concentration for several different cosolutes. Free energies are shown for both (charged) salts (Figure 3A) and net-neutral (Figure 3B) cosolutes. For all cosolutes, except KI, we find a linear dependence of the binding free energy with cosolute concentration.

The action of cosolutes on the reaction can be analyzed in terms of either a difference in the number of associated cosolutes between products and reactants, Δn_s, or a difference in the number of associated waters, Δn_w. The two approaches are necessarily connected through the Gibbs−Duhem equation, relating the concurrent changes in μ_w and μ_s, water and cosolute chemical potential, respectively, due to cosolute addition.

The number of cosolute-excluding waters, Δn_w, released in complexation is related to the change in binding free energy with changes in water chemical potential, μ_w, by[36,37,41,49−51]

$$\Delta n_w = -\frac{d\Delta G°}{d\mu_w} = \frac{55.6}{RT}\frac{d\Delta G°}{dm_s^{osm}} \quad (1)$$

Here T is the absolute temperature, and R the ideal gas constant; 55.6 is the number of moles of water in 1 Kg, and m_s^{osm} is the solute osmolal concentration, a measure of water chemical potential. If CD/AD concentrations are much smaller than cosolute concentration, then $dm_s^{osm} = -(55.6/RT)d\mu_w$.

In the limit of low CD/AD concentrations, Δn_w is also related to Δn_s, the change in the excess number of cosolutes associated with, or preferentially excluded from, CD/AD.[39−41] Again, the link between Δn_s and Δn_w is made using the Gibbs−Duhem relationship in this limit of low CD/AD concentration, $m_w d\mu_w + m_s d\mu_s = 0$, where m_w and m_s are water and solute molal concentrations, respectively. Therefore, we find

$$\Delta n_s = -\frac{d\Delta G°}{d\mu_s} = \Delta n_w \frac{d\mu_w}{d\mu_s} \cong -\frac{m_s \Delta n_w}{55.6} \quad (2)$$

Note that in this limit, Δn_s is also directly related to the difference in Γ_s, the preferential interaction coefficient, between products and reactants, $\Delta\Gamma_s = \Delta n_s$.[39−42,53]

The linearity of the plots shown in Figure 3, even in the limit $m_s \rightarrow 0$, translates using eq 1 into a constant change in the number of cosolute-excluding waters upon binding Δn_w. Conversely, using eq 2, we find that the change in associated cosolute, Δn_s, between reactants and products must vary linearly with cosolute osmolality. We focus here on Δn_w because it remains constant as cosolute concentration is varied. The reactants and products are not single species, but rather a distribution of conformations and geometries, as observed for products by NMR.[27] The measured Δn_w may include changes in the probabilities of these configurations. From the thermodynamic analysis, however, we find that the change in hydration upon association is constant, even at the highest cosolute concentrations we have used, as seen in the linearity of the sets in Figure 3. This indicates that if, indeed, different complex geometries are preferred at different cosolute concentrations, all shed the same number of waters upon complexation.

For salts (Figure 3A), we find that the number of excluded waters depends on the ionic species, reflecting different extents of exclusion from CD/AD interacting surfaces. The extent of exclusion depends more sensitively on the anion than the cation and generally follows the classical Hofmeister series ordering.[44,54,55] For LiCl, KCl, and CsCl, we find that $\Delta n_w \approx -23$ waters are released in complexation; for K_2SO_4, we find $\Delta n_w = -53$ water molecules displaced; for KF and KCl, $\Delta n_w = -23$, and for KBr, $\Delta n_w = -16$. In solutions of KSCN, no change is observed in the association constant, corresponding to $\Delta n_w = 0$. Finally, in KI, a weak nonlinear relationship is found, possibly reflecting association of I_n^- clusters or molecular I_2 with the CD, and subsequent competition with AD for binding.

For the neutral cosolutes shown in Figure 3B, there is also a wide range of exclusion or inclusion reflected in the changes in numbers of cosolute-excluding waters upon complexation.

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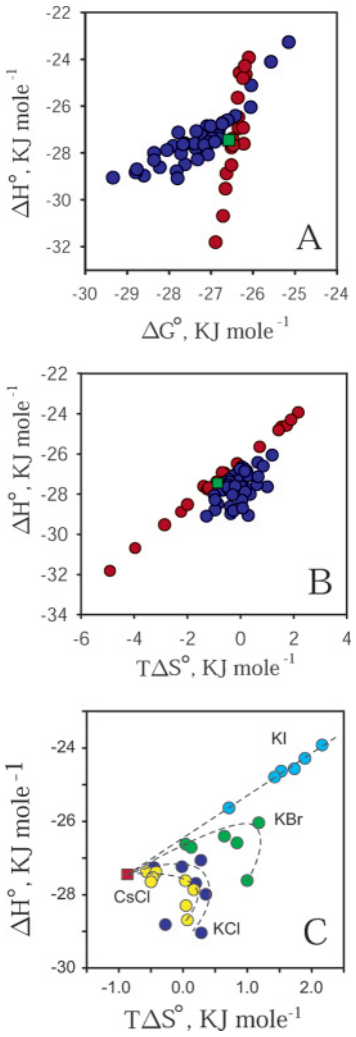

Figure 4. Correlation between (A) heats and free energies of complexation and (B) heats and entropies of association. All cosolutes shown in Figure 3 seem to belong to either of two groups. In blue, KCl, CsCl, LiCl, K_2SO_4, KF, KBr, betaine, and glycine. In red, KSCN, KI, sucrose, and urea. (C) Expanded part of B, showing curved traces in the $\Delta H°-T\Delta S°$ plane for several salts. Colors as for Figure 3; green and red squares correspond to no cosolute addition.

Glycine is most excluded ($\Delta n_w = -24$), while betaine (glycine with a trimethylamine) is most included ($\Delta n_w = +22$). Note that $\Delta G°$ versus osmolal concentration is linear even though betaine and urea preferentially associate with CD/AD, namely, they interact favorably with CD/AD binding surfaces.

In Figure 4, we follow the correlation between the heat release, entropy change, and free energy in different cosolutes. We find that all cosolutes can clearly be associated with one of two cosolute categories, thermodynamically distinct in their mode of action. The first group of cosolutes includes most salts, betaine, and glycine (Figure 4 in blue). Most of the change in binding free energy for these cosolutes is due to a change in heat released upon complexation, while the entropic change is small. Stated in the form $T\Delta S° = \alpha\Delta H° + T\Delta S°_0$,, we find an average of $\alpha = 0.39$ for these cosolutes.

The other group of solutes, including KSCN, KI, sucrose, and urea, has smaller Δn_w values for the binding reaction. However, this weak effect on association constant is the result of large, yet compensating changes in the enthalpy and entropy. In this class, $\alpha = 0.91$ with only a small net change in association free energy with cosolute concentration, indicating a small change in cosolute exclusion−inclusion upon CD−AD binding. Such entropy−enthalpy compensations have been previously attributed to processes where water release is believed to be involved[22] and may possibly be related here to a compensation associated with the release of cavity/surface waters.

Figure 4C shows experimental traces in the $\Delta H°-\Delta S°$ plane for KI, KBr, KCl, and CsCl. While we cannot exclude an experimental artifact in determining $\Delta S°$, these cosolutes seem to trace elliptical curves of varying sizes. Such curves have also been reported in experiments by Eftink et al. for protein−ligand binding and cyclodextrin interacting with a series of different guests.[21,56] Interestingly, we find here a similar relationship between entropy and enthalpy of association when solution conditions are varied by added cosolute as when a series of guests of different sizes but similar morphology are used for complexation.

Osmotic Consequences of Molecular Immersion. By measuring the changes in solution osmolality due to addition of macromolecules, it is possible to determine the extent to which a macromolecule preferentially takes up water from the bathing solution, that is, water unavailable to small "excluded" cosolute.[41,52] Assume that every solute macromolecule is surrounded by n_w cosolute-excluding waters. The addition of m_m moles of (macromolecular) solute to 1 Kg of water (≈55.6 mol) will leave only $55.6 - m_m n_w$ water molecules available for dissolution of any other cosolute. Hence, if a solution contains $m_s°$ moles of cosolute, the *change* in the observed osmolal concentration of cosolute due to an addition of m_m solute molecules will be $\Delta m_s^{osm} \cong m_s° m_m n_w/(55.6 - m_m n_w)$. The corresponding *variation* of change in solution osmolality, Δm_s^{osm}, following addition of a small amount of (macromolecular) solute at concentration m_m to solution with initial cosolute concentration $m_s°$, is related to the number of excluding waters per molecule n_w through

$$\frac{d\Delta m_s^{osm}}{dm_s°} \cong \frac{m_m}{55.6} n_w \qquad (3)$$

Equation 3 assumes that m_m is small enough such that intermacromolecule interactions are negligible. Then, from the differences in numbers of excluding waters in reactants and products, we determine the extent of excluding waters released in association.

Once again, due to the link between solute and water chemical potential imposed by the Gibbs−Duhem relation, we can also follow n_s, the excess/deficit number of cosolute rather than n_w, the corresponding number of waters.[39,41] However, we focus our discussion on n_w, which remains constant over the range of cosolute concentrations studied.

The CD/AD complex formed from a 1:1 molar mixture can be considered as *one macromolecular species* because, at the concentrations used, the amount of unassociated CD/AD in the mixtures is less than 5%. From eq 3, it is apparent that changes in osmolalities depend not only on the number of cosolute-excluding waters (n_w) but also on solute concentration (m_m). However, accuracy in measurements of solution osmolality

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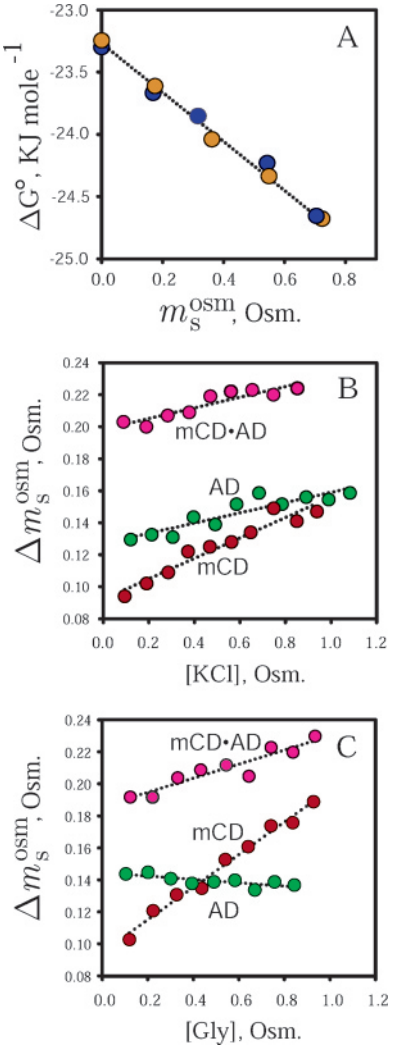

Figure 5. In A, Δn_w is evaluated from changes in association free energies ($\Delta G°$), as determined using ITC, with cosolute osmolal concentration (m_s^{osm}) for mCD showing $\Delta n_w = -40$ for both KCl and glycine. Colors as for Figure 3. In B and C, numbers of cosolute-excluding waters from AD (green), mCD (red), and AD−mCD (magenta), witnessed in the change in solution osmolal concentration (Δm_s^{osm}) with cosolute concentration (eq 3). Cosolutes (B) KCl and (C) glycine. Differences in slopes give changes in cosolute exclusion upon complexation: $\Delta n_w = -43$ in KCl and -31 in glycine.

is limited to ≈2 mOsm, and hence the solubility of β-CD is too low for a precise measurement of osmolality of added cosolute. For this particular measurement, we therefore use a methylated derivative of β-CD, mCD, for which ≈13 hydroxyls (out of 21) per β-CD molecule are randomly methylated. From the correspondence of ITC measurements, performed at low (millimolar) CD/AD concentrations, with osmometry measurements, performed at higher (≈100 mM) concentrations, we conclude that the same association reaction is being probed in both experiments, and competing interactions among CD or AD are negligible.

Figure 5A shows the complexation free energy for mCD/AD in the presence of glycine or KCl at different osmolal concentrations, as derived from ITC. From the slope, we estimate $\Delta n_w = -40 \pm 2$ for both cosolutes. We then immerse small amounts of AD, mCD, or AD−mCD complexes in a cosolute containing bathing solution and measure the effect on solution concentration using vapor pressure osmometry. Figure 5B,C shows the change of measured osmolality upon addition of CD, AD, or an equimolar mixture of the two to a solution of cosolutes: either KCl or glycine. Using eq 3, we derive numbers of excluding waters from the slopes of Δm_s^{osm} with cosolute osmolality.

Though the change in number of excluding waters upon complexation (Δn_w) for KCl and glycine is the same (within experimental error), the number of excluded waters from each of the molecular species is different. Glycine is highly excluded from mCD ($n_w = 61 \pm 4$), but it is almost indifferent toward AD ($n_w = -5 \pm 4$). In contrast, KCl is excluded to a similar extent ($n_w = 36 \pm 4$ and 25 ± 4, respectively) from both mCD and AD. The CD/AD complex excludes KCl and glycine from 18 ± 4 and 25 ± 4 hydrating waters, respectively. The net change for the two cosolutes is $\Delta n_w = -43 \pm 7$ for KCl and $\Delta n_w = -31 \pm 7$ for glycine. Numbers of released waters evaluated using ITC (Figure 5A, $\Delta n_w = -40 \pm 2$) agree reasonably well with those determined from the osmotic pressure measurements. The correspondence in numbers obtained from both ITC and osmometry confirms that both approaches indeed probe the release of cosolute-excluding waters.

It is possible that differences in exclusion of KCl and glycine from the individual CD/AD species are due to differences in interactions of cosolute with surfaces that remain exposed after complex formation (such as CD exterior or the charged carboxylate group on AD), while exclusion from the nonpolar interacting surfaces is similar. Only cosolute that is excluded from interacting surfaces probes water release; exclusion/inclusion from other parts of the molecule is likely unaffected by complexation.

Discussion

Osmotic Stress and Water Release. The osmotic stress technique is becoming an increasingly popular tool for investigating changes in hydration accompanying macromolecular reactions.[28,37,49−51] The approach offers several opportunities. It is widely appreciated that water plays an important role in determining binding energetics, but few methods are available for measuring water release coupled to association. Utilizing osmotic pressure to act on a difference in the number of solute-excluding waters associated with products and reactants is a powerful and practical method for enhancing the stability of complexes. From the dependence of Δn_w on the chemical natures of the probing solute and macromolecular surface, we learn about the nature of the physical interactions between molecules. Last, crowded with salts, sugars, amino acids, and other macromolecules, such as DNA and proteins,[47,57] the intracellular milieu is far different from the dilute aqueous conditions typically used to study the enzymatic, recognition, and assembly reactions that occur in living systems. The osmotic stress technique illustrates how important such "crowding" can be.

By changing the concentration of a cosolute that is excluded from the macromolecule, we evaluate changes in the number of cosolute-excluding waters upon association. Here, we have studied the role of water release in a convenient model system in which two molecules, cyclodextrin (CD) and adamantane carboxylate (AD), present complementary hydrophobic surfaces and associate with specificity.[58]

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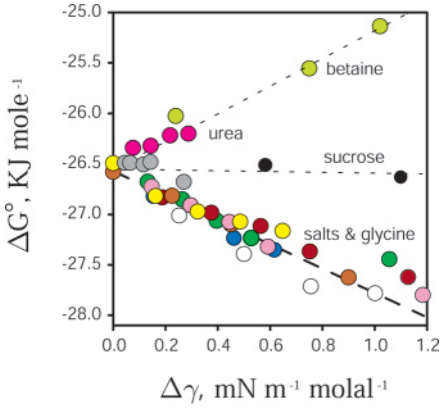

Figure 6. Changes in association free energy correlated with the bathing solution's change in air−water interfacial tension (compared with that of pure water) for different cosolutes. Colors as for Figure 3.

Using ITC, with corroboration from osmometry, we find for many cosolutes that ≈15−25 cosolute-excluding water molecules are released upon complex formation with β-CD. This is consistent with a release of all waters from the cyclodextrin cavity (≈11 waters in the crystal) and additional waters from the adamantane surface.

All cosolutes we have used fall into one of two classes. In the first, which includes many salts and glycine, the contribution to the binding free energy from solute−macromolecule interactions is dominated by enthalpy. For the second group of cosolutes, which are found to have only a weak net inclusion/exclusion, the enthalpic contribution is also large but is almost fully compensated by the change in entropy.

Surface Waters and Surface Tension. One model that has been suggested to account for how cosolutes affect association was first proposed by Sinanoglu and Abdulnur.[59] In that model, cosolute (or solvent) effects were associated with the work needed to create an empty cavity in solution, that is, to surface tensions.[20,40,53,60] To test this idea in CD/AD association, we correlate changes of association strength with surface tensions as measured at the air−water interface for salt and other cosolute solutions.

Figure 6 shows, for most of the cosolutes used in this study, the changes in association free energy as a function of experimentally known changes in air−water interfacial tension.[44,61−65] Strikingly, for many salts, all data fall on a single line. The slope of this line represents an effective surface area change upon complexation of ≈200 Å^2. If we assume a cosolute-excluding water layer one molecule thick (≈3 Å) and a volume of 30 Å^3 per water molecule, we conclude there are ≈20 waters released during the inclusion process. This matches the estimate from the responses to osmotic stress. For KCl and glycine, however, the correlation with surface tension only holds for the difference in waters for the reaction and not for the waters included with the individual species (Figure 5). The correlation seems to reflect only the surfaces that interact in the complex.

Interestingly, the reaction heat capacity for CD/AD complexation was previously found to be −398 J mol^{-1} K^{-1}.[20] Using the range of reported estimates for the heat capacity associated with the burial of exposed hydrophobic surfaces,[16−19] and assuming that CD/AD interacting surfaces are fully hydrophobic in nature, we find a corresponding reduction of 180−340 Å^2 in surface exposed to solution upon complexation. Assuming the existence of a surface layer of cosolute-excluding water 3 Å thick implies a release of 18−34 waters upon complexation. Perhaps fortuitously, this estimate also agrees with the numbers found using the osmotic stress analysis.

For other (predominantly neutral) cosolutes, a correlation does not hold between air−water interfaces and changes in complex stability. We may infer that these cosolutes interact differently with the macromolecule surface than with an air−water interface.

Interactions Involved in Cosolute Exclusion. In terms of the Gibbs adsorption isotherm,[66] the excess or deficit of cosolutes from the interacting surfaces is directly related to changes in surface stability with varied cosolute activity. The excesses or deficits depend on interactions of the solvated cosolute and macromolecule.

We may ask "what interactions are responsible for cosolute exclusion from CD/AD"? Alternatively, we may ask "in what way do surface waters pose a less favorable environment (solvent) to cosolutes"?

One possible origin of exclusion is through steric "excluded volume" interactions between cosolute and CD/AD. Because these forces are manifested due to loss in translational entropy near a macromolecule, no heat evolution is expected. However, experimentally, all cosolutes show a large enthalpic contribution to the change in complexation free energy (Figure 4). While we cannot rule out this as a contributing interaction, we can conclude that crowding due to steric solute−cosolute interactions is not the main contributor to the overall free energy change due to cosolute.

Electrostatic "image charge" interactions of ions with the nonpolar CD/AD surfaces are another possible source of preferential exclusion. By the reasoning of Onsager and Samaras,[67] ions approaching an interface, going from a high to lower dielectric material, are repelled due to loss of favorable interactions with the high dielectric medium. This unfavorable energy competes with the translational entropy of an ion to form an ion-excluded region, typically a few angstroms thick. While the temperature dependence of the original Onsager and Samaras model does not match our findings, and the cyclodextrin cavity and adamantane are not simple nonpolar surfaces, it seems reasonable to expect that such an electrostatic repulsion could contribute to the net exclusion of ions.

What then can account for the differences found among different salts? In particular, the more polarizable ions, such as Br$^-$ and SCN$^-$, appear here, as in many other experiments, to be less excluded from the macromolecular interface than, say, Cl$^-$ and even more so SO_4^{2-}.[43,44,68−75] Observations of similar

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trends go back to the seminal experiments on protein precipitation by different salts that led to the so-called Hofmeister series.[54] It has been proposed that interacting hydrocarbon surfaces (rather than air) introduce the likely van der Waals-type attraction of more polarizable ions to higher index of refraction hydrocarbon.[55,76]

The remarkable correspondence between free energy changes of CD/AD binding due to solute exclusion and surface tensions at air−water interfaces seen in Figure 6 for many of the cosolutes suggests that these cosolutes are interacting with interfacial water that is structured differently from the bulk due to the presence of an air or CD/AD surface. Irrespective of the detailed water structure, this asymmetry will present a locally inhomogeneous dipolar layer of waters to cosolutes. More polarizable ions can "dissolve" better in such a layer, due to an added favorable dipole to induced-dipole interaction.[5,55,77,78] Such an argument could imply a generality of the effect, insensitive to the type of interface formed.[45,53,59] The nature of the surface in contact with water would then be of secondary importance.

Finally, the close interaction of solutes and surfaces necessarily alters the structuring of water around each. The energetics connected with this restructuring of water as two surfaces approach has been suggested as the basis for the common exponential force seen between many macromolecules, both charged and uncharged, at spacings closer than ≈10 Å.[79,80] The extracted spatial dependences for the exclusion of nonpolar alcohols from DNA and of salts and polar solutes from hydroxypropyl cellulose show the same type of exponential behavior as that associated with the postulated hydration force.[81−83] Differences in water structuring around cosolute may, for example, explain the differences in the extent of exclusion/inclusion from CD/AD of glycine with its amine group versus the trimethylated amine analogue, betaine. It is also important to consider water structuring involved in the hydration of cosolutes themselves. Indeed, ions in the Hofmeister series tend to structure water very differently.

Concluding Remarks

Perhaps most important, the results derived using the different approaches are commensurate, enabling a convergence of different perspectives and languages for speaking about interactions of macromolecules in solution. We learn that the solvating power of water around and within cyclodextrin and adamantane differs from that of bulk water, consonant with what has been observed in many specifically interacting biomacromolecules.[84] These waters could be responsible for cosolute exclusion due to their interfacial ordering properties. If so, these waters not only exclude many cosolutes but also preferentially interact with salts to different extents, correlating with ionic polarizability. In concert, direct repulsive solute−surface interactions may also contribute to creating a preferential hydration layer.

By subjecting CD and AD to cosolutes, we show that the strength of their association can be modulated and controlled. Important implications follow because in all technological and pharmacological applications, the systems considered are not pure aqueous solutions, but rather physiological milieus, crowded with small cosolutes and other macromolecules that will affect the binding.[85,86] In fact, cosolute-containing formulations have been proposed as a way to enhance the association of CD with guest molecules.[87] Understanding the preferential interactions of cosolutes with CD will aid in the rational development of effective formulations.

Experimental Section

Cyclodextrins (Fluka) were dried overnight in vacuum; then stock solutions were made by weight. Adamantane carboxylic acid (Fluka) was dissolved in 0.02 M phosphate buffer solution, pH 6.9 (for low concentrations), or titrated with NaOH until fully dissolved, and then buffered in the same way (for high concentration). All chemicals were used with no further purification.

Microcalorimetry. Isothermal titration calorimetric measurements were made at 30 °C using a VP−ITC microcalorimeter (MicroCal). In each experiment, 40 successive 7 μl injections of 5.5−6.5 mM CD solutions were mixed into the thermostated cell containing 0.4−0.5 mM AD solution; solutions were stirred at 300 rpm. Injections lasted 10 s and were spaced at 3.5 min intervals. The heat release associated with AD and CD dilution was measured separately and did not substantially change the calculated thermodynamic properties. Solutions were in 0.02 M Na phosphate buffer at pH 6.9. Both AD and CD solutions contained an additional cosolute, both with the same osmolality.

Equilibrium constants, heats, and entropies of reaction were evaluated using standard MicroCal Origin software procedures. All fits to the data were consistent to within 3% with a 1:1 molar complexation ratio. Attempted fits to other possible complex ratios did not improve the overall fit, supporting the simplest assumption of 1:1 binding under all studied conditions. Presented results are averages of 2−8 repeats and include an uncertainty, expressed as standard deviations, of no more than ±1% for heats of reaction and ±0.5% for the free energies. As detailed in the Results section, changes in numbers of hydrating waters were evaluated from linear least-squares fits to evaluated free energies versus cosolute concentrations. The fits involve an error of, at most, ±5 waters.

Osmolalities of cosolute solutions were measured separately on a Wescor 5520 vapor pressure osmometer.

Osmometry. The osmolality of a series of solutions containing cosolute (KCL or glycine) and water, measured on a Wescor 5520 osmometer, was compared with osmolalities of solutions with the same cosolute molality, but with added solute (AD, CD, or an equimolar mixture of the two). Solute concentrations were in the 70−200 mOsm range and that of cosolute was 50−1000 mM.

Acknowledgment. We thank Horia Petrache for useful comments on the manuscript.

JA045541T

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